PHORBOL ESTERS AND CALCIUM IONOPHORE CAN PRIME MURINE PERITONEAL MACROPHAGES FOR TUMOR CELL DESTRUCTION

Murine macrophages from sites of inflammation develop toward tumoricidal competence by exposure to a macrophage-activating factor such as interferon-γ (IFN-γ). To explore the biochemical transductional events initiated by IFN-γ, peritoneal macrophages from C57BL/6J mice elicited by various sterile irritants were treated *in vitro* with two pharmacologic agents that mimic the action of certain second messengers. Phorbol myristate acetate (PMA) and the ionophore A23187 cooperatively reproduced the ability of IFN-γ to prime macrophages for tumoricidal function. Neither agent alone was able to prime macrophages. The two agents acted on the macrophages, and target susceptibility to killl was not altered by PMA and A23187. Only active phorbol esters, which are known to bind and stimulate protein kinase C, were able to cooperate with A23187 to induce priming. A cell-permeable synthetic diacylglycerol (sn-1,2-dioctanoyl glycerol) could also prime for cytolysis. In the presence of PMA, A23187, and EGTA, addition of Ca^{++} was sufficient for priming, whereas the addition of Mg^{++} was much less efficient. Priming by IFN-γ, however, was not blocked by EGTA. Efflux of $^{45}Ca^{++}$ from preloaded cells was significantly increased by A23187 and by IFN-γ. Quin-2/AM, an intracellular chelator of Ca^{++}, blocked priming by IFN-γ. In summary, the data suggest that priming of macrophages for tumoricidal function by IFN-γ involves, at least in part, alterations in protein kinase C and in levels of intracellular Ca^{++}.

Somers, S. D., Weiel, J. E., Hamilton, T. A., and *Adams, D. O.*

The Journal of Immunology **136**(11):4199-4205, June 1, 1986.

Other support: U. S. Public Health Service.

From the Department of Microbiology/Immunology, Duke University Medical Center, Durham, NC.

EFFECTS OF BACTERIAL LIPOPOLYSACCHARIDE ON PROTEIN SYNTHESIS IN MURINE PERITONEAL MACROPHAGES: RELATIONSHIP TO ACTIVATION FOR MACROPHAGE TUMORICIDAL FUNCTION

Early biochemical events in the response of murine peritoneal macrophages to bacterial lipopolysaccharide (LPS) had been examined (i.e., 0-4 hr after initiation of treatment). At concentrations of 10 ng/ml or less, LPS stimulated the new or enhanced synthesis of a series of at least six polypeptides of 85, 80, 75, 65, 57, and 38 kD. This effect was dependent upon the lipid A moiety of LPS as lipid A itself could induce the changes and the effect of LPS could be blocked by inclusion of polymixin B sulfate in the culture medium. The effect was specific for LPS in that other endotoxin-free agents known to alter macrophage physiology could not produce the same changes. The time course of LPS stimulation of macrophage protein synthesis was remarkable in that the synthesis of all six proteins was transient even in the continued presence of LPS, being first detected approximately 1 hr after exposure and no longer apparent by 8-10 hr after treatment was initiated. Furthermore, both pulse-chase and cumulative radiolabeling studies indicated that at least two of the proteins (85 and 35 kD) were short-lived and did not accumulate in LPS-treated cells, suggesting the possibility that they participate in a regulatory rather than a functional role. Macrophage tumoricidal activation in-

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volves cooperation in response to two independent signals; interferon gamma and LPS. Pretreatment of macrophages with interferon gamma increased the sensitivity of macrophages to LPS-stimulated protein synthesis by one to two orders of magnitude documenting such cooperativity in molecular terms. The LPS-induced stimulation of specific protein synthesis could be reproduced by treatment of macrophages with heat killed *Listeria monocytogenes*, a gram-positive, endotoxin-negative bacterial stain which has been shown to substitute effectively for LPS in macrophage tumoricidal activation. Furthermore, reversible inhibition (i.e., treatment with cycloheximide) of protein synthesis during LPS treatment abrogated the acquisition of tumoricidal function. These results identify an early biochemical response to LPS which may be a necessary component of the intracellular transduction of signals which regulate macrophage functional development.

Hamilton, T. A., Jansen, M. M., Somers, S. D., and *Adams, D. O.*

Journal of Cellular Physiology **128**:9-17, 1986.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

REGULATION OF RESPIRATORY BURST IN MURINE PERITONEAL MACROPHAGES: DIFFERENTIAL SENSITIVITY TO PHORBOL DIESTERS BY MACROPHAGES IN DIFFERENT STATES OF FUNCTIONAL ACTIVATION

Activation of macrophages either *in vivo* or *in vitro* can modulate the capacity to generate and secrete reactive oxygen intermediates including H_2O_2 and O_2^-. Thus, the cellular and biochemical components requisite for execution of the respiratory burst must be regulated during the activation process. In the present report, we have examined murine peritoneal macrophages in different stages of activation for their sensitivity to stimulants of respiratory burst known to activate protein kinase c (i.e., phorbol dibutyrate or diacylglycerol). The results demonstrated that more highly activated macrophages showed, in addition to greater magnitude of H_2O_2 or O_2^- production, a two- to fourfold greater sensitivity to these stimuli. While more active macrophages also exhibited a higher rate of H_2O_2 secretion, the time at which secretion was measured did not account for or modulate the heightened sensitivity. The increased sensitivity to stimulation was dependent upon the stage of activation and not on the agent used to elicit the macrophages. Increased sensitivity of the more active macrophage populations was also seen when physiologic stimuli (i.e., insoluble immune complexes or unopsonized zymosan) were used. These findings indicate that macrophage activation of H_2O_2 secretion modulates the sensitivity to stimulation such that more H_2O_2 is produced in a shorter time and at a lower concentration of stimulus, thereby heightening the inflammatory response in several independent ways. Because all the stimuli employed in the present study have in common the ability to activate protein kinase c (either directly or indirectly), the data also suggest that this form of macrophage activation may involve, at least in part, modulation of the stimulus-response coupling mechanism which utilize this enzyme.

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

Johnston, P. A., *Adams, D. O.*, and Hamilton, T. A.

Cellular Immunology **100**:400-410, 1986.

Other support: U. S. Public Health Service.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

TREATMENT OF MURINE PERITONEAL MACROPHAGES WITH BACTERIAL LIPOPOLYSACCHARIDE ALTERS EXPRESSION OF c-FOS AND c-MYC ONCOGENES

Expression of the c-fos, c-myc, and c-fms proto-oncogenes has been studied in thioglycollate-elicited murine peritoneal macrophages after exposure to lipopolysaccharide (LPS). After incubation with LPS (20 ng/ml), a transient and rapid induction of the expression of c-fos and c-myc oncogenes could be observed, whereas the RNA levels for c-fms were not affected. Treatment with lipid A, the active moiety of the LPS molecule, increased the c-fos and c-myc expression to a comparable degree. Similar induction of c-fos and c-myc was observed after treatment with phorbol myristate acetate, suggesting that this effect of LPS on murine macrophages might be mediated through stimulation of protein kinase C. Under similar experimental conditions, LPS treatment of macrophages did not trigger DNA synthesis. Treatment with LPS blocked DNA synthesis in macrophages treated with L cell-conditioned medium containing colony-stimulating factor. Thus changes in c-fos and c-myc expression may be elements in the complex series of biochemical events that contribute to macrophage activation and are not necessarily related to induction or priming for cellular proliferation.

Introna, M., Hamilton, T. A., Kaufman, R. E., and *Adams, D. O.*

The Journal of Immunology **137**(8):2711-2715, October 15, 1986.

From the Department of Pathology, Duke University Medical Center, Durham, NC.

ANALYSIS OF DEFICIENCIES IN IFN-γ-MEDIATED PRIMING FOR TUMOR CYTOTOXICITY IN PERITONEAL MACROPHAGES FROM A/J MICE

The functional and biochemical responses of macrophages derived from the A/J mouse strain to IFN-γ have been studied. As compared to macrophages obtained from C57BL/6 strain mice, cells from mice of the A/J strain are deficient in their response to IFN-γ for acquisition of tumoricidal competence. This deficiency was not due to reduced expression of surface receptors for IFN-γ or to altered affinity of the receptor for its ligand. IFN-γ recently has been shown to enhance the potential activity of protein kinase C (PKc) and to modulate the efflux of intracellular Ca^{++} in macrophages from C57BL/6 mice. Neither of these two biochemical changes was induced in macrophages derived from A/J mice. Functional competence could, however, be pharmacologically induced in both C57BL/6- and A/J-derived macrophages by combined treatment with an ionophore plus phorbol myristic acetate, which increase intracellular Ca^{++} and stimulate PKc, respectively. Although the exact nature of the deficit in A/J

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strain mice has not been defined, the present findings indicate that it lies between the expression of receptor and the modulation of PKc activity and Ca^{++} levels. Furthermore, the data provide support for the notion that these molecular changes are important components of the stimulus-response coupling process in IFN-γ-mediated activation of macrophages.

Hamilton, T. A., Somers, S. D., Becton, D. L., and *Adams, D. O.*

The Journal of Immunology **137**(10):3367-3371, November 15, 1986.

Other support: U. S. Public Health Service, Eli Lilly Research Laboratories and the Elsa V. Pardee Foundation.

From the Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, NC.

VIRAL INFECTION OF VASCULAR ENDOTHELIAL CELLS ALTERS PRODUCTION OF COLON-STIMULATING ACTIVITY

Viral infections in humans are frequently associated with granulocytopenia and/or granulocytosis. Such changes in myelopoiesis could result from infection of the granulocyte-macrophage colony-forming cell (CFC-GM) or changes in the production of colony-stimulating activity (CSA). Endothelial cells are a known source of CSA and may be transiently or persistently infected during a number of viral infections, including infection with herpes simplex virus type I (HSV-I) and measles virus. Therefore, we examined the effect of endothelial cell infection with these two viruses on the production of CSA. Uninfected passaged endothelial cells produce CSA when stimulated by the continual presence of a factor present in medium conditioned by peripheral blood monocytes (MCM). Within 4 h of infection with HSV-I, endothelial cells no longer produced CSA in response to MCM. In contrast, measles virus infection induced CSA production by passaged endothelial cells even in the absence of MCM. Measles virus-induced CSA production was maximal at 24 h and required the presence of live virus within the endothelial cells. The effects of HSV-I and measles virus on CSA production were not dependent on alterations in the production of α- or γ-interferon by the infected endothelial cells. Infection with HSV-I did not stimulate endothelial cells to release any detectable interferon. In contrast, the supernatants of the measles-infected cells contained only β-interferon, a known inhibitor of CFC-GM development. These studies suggest that CSA production by endothelial cells is directly altered by infection with HSV-I and measles virus. An alteration in CSA production might contribute to changes in myelopoiesis that frequently accompany viral infection in humans.

Gerson, S. L., Friedman, H. M., and *Cines, D. B.*

Journal of Clinical Investigation **76**:1382-1390, 1985.

Other support: National Institutes of Health and the Sohio Foundation.

From the Division of Hematology-Oncology, University Hospitals of Cleveland, Cleveland, OH; and the Divisions of Infectious Diseases and Hematology-Oncology, Hospital of the University of Pennsylvania, Philadelphia.

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

ANTIBODY AND IMMUNE COMPLEXES INDUCE TISSUE FACTOR PRODUCTION BY HUMAN ENDOTHELIAL CELLS

Patients with systemic lupus erythematosus (SLE) have an increased incidence of arterial and venous thromboses. The mechanism by which thromboses develop in these patients is unknown. We had previously observed that the sera of patients with SLE contain antibodies and immune complexes that can bind to endothelial cells. Because endothelial cells can synthesize tissue factor, a potent activator of coagulation, we studied the effect of IgG complexes and sera from patients with SLE on the production of tissue factor by these cells. Human umbilical venous endothelial cells incubated with heat-aggregated IgG (HA-IgG) (0.5 to 4.0 mg) elaborate procoagulant activity in a dose-dependent manner. All procoagulant activity was found in the particulate cell fraction, and none was secreted into the medium. Maximum expression of procoagulant activity required 6 to 8 hr, and its production was totally inhibited by the addition of cyclohexamide or actinomycin D. The presence of gel-filtered platelets augmented production of procoagulant activity by endothelial cells stimulated by HA-IgG. Endothelial cell procoagulant activity was not inactivated by diisofluoropropylphosphate, required the presence of Factor VII for its expression, and was neutralized by a specific anti-tissue factor antibody. Endothelial cells incubated with sera from 14 of 16 patients with SLE produced increased amounts of tissue factor compared with 21 normal sera ($p < 0.025$). Fractions of two SLE sera containing monomeric IgG, IgA, or IgM, as well as fractions containing IgG complexes, each stimulated endothelial cells to produce more tissue factor than similar fractions prepared from two normal sera. These studies demonstrate that endothelial cells will produce the procoagulant tissue factor after exposure to anti-endothelial cell antibodies or IgG-containing immune complexes. The production of tissue factor by endothelial cells at sites of immune vascular injury may play a role in the development of thromboses in patients with SLE.

Tannenbaum, S. H., Finko, R., and *Cines, D. B.*

The Journal of Immunology **137**(5):1532-1537, 1986.

Other support: National Institutes of Health.

From the Hematology-Oncology Section, Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia.

ANTIGENIC ANALYSIS OF HEMATOPOIESIS. V. CHARACTERIZATION OF My-10 ANTIGEN EXPRESSION BY NORMAL LYMPHOHEMATOPOIETIC PROGENITOR

The My-10 glycoprotein is an hematopoietic cell surface antigen expressed specifically by undifferentiated (blast) cells, constituting 1%–4% of normal adult bone marrow leukocytes. We used several immunological and in vitro culture methods to analyze the expression of this unique antigen on a variety of lymphohematopoietic progenitor cells. Colony-forming cells (CFC) for granulocyte-monocyte colonies (CFC-GM) and erythroid colonies (BFU-E) were predominantly My-10 positive. CFC with higher proliferative potential were more strongly My-10 positive than CFC with lower proliferative potential, and those for mixed-lineage and blast cell colonies were even more uniformly My-10 positive. Cells maintaining CFC-GM number in short-term marrow culture (pre-CFC) were found to be My-10 positive, as were lymphoid

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precursors defined by their content of intranuclear terminal deoxynucleotidyl transferase. More mature erythroid precursors (CFU-E) were heterogeneous for antigen expression and lost My-10 antigen progressively, in parallel with advancing maturational stage. The My-10 antigen permits rapid identification and purification of hematopoietic progenitor cells for further study or potential clinical application. The disappearance of the My-10 antigen, moreover, may be a probe for differentiation-linked cellular events.

Strauss, L. C., Rowley, S. D., La Russo, V. F., Sharkis, S. J., Stuart, R. K., and *Civin, C. I.*

Experimental Hematology **14**:878-886, 1986.

Other support: National Institutes of Health and the American Cancer Society.

From The Johns Hopkins Medical Center, Divisions of Pediatric Oncology and Experimental Hematology, Johns Hopkins University School of Medicine, Baltimore, MD.

SIALYLATED GLYCOLIPID ANTIGENS ON HUMAN LEUKEMIC CELL LINES

Many granulocyte-specific mouse monoclonal antibodies recognize the carbohydrate sequence 3-fucosyllactosamine, Galβ1–4[Fucα1–3]GlcNAc, which occurs in cell-surface glycolipids and glycoproteins. In general, these antibodies bind to blast cells from most patients with acute myeloblastic leukemia, but not to those with acute lymphocytic leukemia. Neuraminidase treatment, however, increases exposure of this antigen on both myeloid and lymphoid cells. In the present study, the glycolipids from 13 lymphoid and nonlymphoid human cell lines were examined for the presence of unsialylated and sialylated 3-fucosyllactosamine sequences using a thin-layer chromatography immunostaining method. Nine of the cell lines were also tested by indirect immunofluorescence both before and after neuraminidase treatment. None of the six B-cell and T-cell lines had detectable neutral or sialylated glycolipid antigen. In contrast, six out of seven and five out of seven nonlymphoid cell lines had neutral and sialylated glycolipid antigens, respectively. These results agreed, in general, with those found by indirect immunofluorescence. They also represent the first direct demonstration of these sialylated glycolipids on human leukemic cells. Thus, in some cases increased antibody binding to neuraminidase-treated cells can be explained by the presence of sialylated glycolipid antigen.

Spitalnik, S. L., Spitalnik, P. F., *Civin, C. I.*, Ball, E. D., Schwartz, J. F., and Ginsburg, V.

Experimental Hematology **14**:643-647, 1986.

Other support: National Institutes of Health.

From the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; the Division of Pediatric Oncology, The Oncology Center, Johns Hopkins University School of Medicine, Baltimore, MD; and the Department of Medicine, Dartmouth Medical School, Hanover, NH.

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IN VITRO RELEASE OF α_1-ACID GLYCOPROTEIN RNA SEQUENCES SHOWS FIDELITY WITH THE ACUTE PHASE RESPONSE *IN VIVO*

The acute phase reaction of rat liver to subcutaneous turpentine challenge results in a 20- to 100-fold increase in α_1-acid glycoprotein (αAGP) mRNA. We utilized this response to establish conditions appropriate for study of RNA transport *in vitro* using hybridization with ^{32}P-labeled exon and intron αAGP sequences. Contamination of nuclear preparations by membrane-absorbed cytoplasmic RNA was eliminated by detergent-rinsing. The *in vitro* incubation conditions that most reflected the *in vivo* state required RNase inhibitor (purified from placenta), polyvinylpyrrolidone to prevent nuclear swelling, and addition of ATP. Under these circumstances, αAGP sequences were transported only from turpentine-stimulated preparations, were found only in poly(A)+ RNA, and were the same size as authentic cytoplasmic mRNA. Omission of polyvinylpyrrolidone resulted in release of some αAGP sequences in smaller, more heterogeneous poly(A) RNA, and leakage of some αAGP sequences was observed from control preparations. Omission of ATP resulted in restriction of mature αAGP mRNA to the nucleus. In contrast to αAGP mRNA, transport of albumin mRNA was decreased 3-4X in turpentine-treated preparations. The largest αAGP intron was not found in RNA transported from treated nuclei in complete medium. The intron-containing fragments remained in the nucleus, largely in poly(A) RNA of a size consistent with free intron. Some hybridization of intron sequences was observed with cytoplasmic and nuclear membrane-associated poly(A)+ RNA preparations which may represent 3′-processing catabolites; leakage of these sequences was considerably greater in the absence of PVP. On the basis of densitometric estimates, a 5-fold increase in the amount of αAGP exon sequences was observed in nuclear RNA, comparing treated with control animals, but transport of αAGP exon sequences was detectable only from treated nuclei, indicating at least a 50-fold increase in abundance of αAGP sequences. This suggests that a selective gating mechanism may be operative at the level of posttranscriptional nucleocytoplasmic transport during induction of αAGP in the acute phase response.

Clawson, G. A. et al.

Molecular Biology Reproduction **11**:163-172, 1986.

Other support: National Institutes of Health and the Academic Senate, University of California at San Francisco.

From the Department of Pathology, University of California School of Medicine, San Francisco.

JH$_1$ PEPTIDE INDUCES ANTIBODIES TO A COMMON IMMUNOGLOBULIN DETERMINANT AS DETECTED BY CELL-BINDING ANALYSIS

In order to more accurately determine the distribution of antigenic determinants detected by antisera to hypervariable-region and JH$_1$ peptides, we measured the frequency of lymphocytes stained with these sera by flow cytometry. None of the sera specific for HV1, HV2 or HV3 peptides stained significant numbers of lymphocytes, but those specific for JH$_1$ reacted with nearly all B-cells.

Seiden, M. V., Srouji, A., *Clevinger, B.*, and Davie, J. M.

Molecular Immunology **23**(2):125-129, 1986.

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Other support: U. S. Public Health Service.

From the Department of Microbiology and Immunology, School of Medicine, and Division of Biomedical Sciences, School of Dental Medicine, Washington University, St. Louis.

EFFECT OF HUMAN SERUM AND SOME OF ITS COMPONENTS ON NEUTROPHIL ADHERENCE AND MIGRATION ACROSS AN EPITHELIUM

The effect of human serum and some of its components on the process of transepithelial migration of human neutrophils was investigated in an *in vitro* system. 10% autologous serum caused an increase in neutrophil adherence to and migration across canine kidney epithelial cells. This increase in neutrophil binding also occurred if the epithelium but not the neutrophils had been preincubated with serum. The binding was lost if the serum was either preabsorbed over the kidney epithelium before use or heat inactivated. Indirect immunofluorescence studies indicated that IgG, IgM, and a component of C3 bound to the epithelial surface, whereas IgA, IgE, or C5a were not detectable. The majority of epithelial cells were immunofluorescent, however epithelial cells with varying degrees of reactivity were also apparent and ~5% of the epithelial cells did not bind IgG, IgM, and C3. When epithelia were simultaneously tested for the presence of either IgG, IgM, or C3, and bound neutrophils, the few epithelial cells which did not bind IgG or IgM also did not bind C3 or neutrophils. Studies with monoclonal antibodies against Fc and C3 receptors indicate that neutrophil adherence to the epithelial surface was mediated predominately by the receptors for C3b and C3bi. In response to a chemotactic gradient, bound neutrophils were able to detach and migrate across the epithelium. A separate heat-stable factor(s) in serum was able to increase neutrophil migration across the epithelial monolayer. This factor acted independently of the factors which caused the increase in neutrophil binding as the increase in neutrophil migration also occurred under conditions (preabsorption over the kidney epithelium or heat inactivation) that prevented the increase in neutrophil binding. The increase in neutrophil migration may be caused by the permeability-increasing properties of this factor as both serum and heat-inactivated serum lowered the transepithelial electrical resistance an average of 38 and 35%, respectively, in 40 min. Upon removal of serum or heat-inactivated serum, the resistance returned 100 and 81%, respectively, in 5 h.

Cramer, E. B., et al.

The Journal of Cell Biology **102:**1868-1877, 1986.

Other support: National Institutes of Health and the New York Heart Association.

From the Department of Anatomy and Cell Biology, State University of New York, Downstate Medical Center, Brooklyn.

ALL HUMAN MONOCYTES HAVE THE CAPABILITY OF EXPRESSING HLA-DQ AND HLA-DP MOLECULES UPON STIMULATION WITH INTERFERON-γ

We have simultaneously studied expression of all three classes of human Ia (HLA-DR, DP, and DQ) on normal human B cells and monocytes by using two-color

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immunofluorescence and flow cytometry. Expression was investigated on freshly isolated cells and after incubation of cells for 48 and 96 hr in interferon-γ (IFN-γ). All freshly isolated B cells express high levels of DR, DQ, and DP, and these levels are unchanged by incubation with IFN-γ for 48 hr and 96 hr. In contrast, freshly isolated monocytes are on the average 91% DR^+, 32% DQ^+, and 15% DP^+. Incubation with IFN-γ increases Ia expression on Mϕ to 98% DR^+, 75% DQ^+, and 58% DP^+ at 48 hr, with virtually all cells becoming positive for all three Ia antigens at 96 hr. Furthermore, after the 96-hr incubation, antigen density increases 10-fold for DR, 15-fold for DQ, and 15-fold for DP in monocytes to reach levels of expression comparable with B cells. These studies demonstrate that all peripheral blood monocytes have the capacity to become HLA-DQ and HLA-DP positive; IFN-γ regulates expression of all three classes of human Ia in monocytes; and IFN-γ does not significantly modulate Ia expression in B cells.

Gonwa, T. A., Frost, J. P. and *Karr, R. W.*

The Journal of Immunology **137**(2):1-6, 1986.

Other support: The Veterans Administration.

From the Veterans Administration Medical Center and the Department of Internal Medicine, University of Iowa, Iowa City.

GAMMA INTERFERON AND 5-AZACYTIDINE CAUSE TRANSCRIPTIONAL ELEVATION OF CLASS I MAJOR HISTOCOMPATIBILITY COMPLEX GENE EXPRESSION IN K562 LEUKEMIA CELLS IN THE ABSENCE OF DIFFERENTIATION

We studied the effects of gamma interferon (IFN-γ) on HLA class I gene expression, differentiation, and proliferative capacity of K562 human leukemia cells. In the uninduced state, K562 cells show little or no class I gene expression but actively express the erythroid-specific γ-globin gene as well as genes associated with cell proliferation, including the transferrin receptor, c-*myc*, and α-actin genes. At both the surface protein and mRNA levels, IFN-γ induces class I and β_2-microglobulin gene expression, but does not alter the expression of the γ-globin, transferrin receptor, c-*myc*, or α-actin genes. A 10-fold maximal induction of both class I surface protein and mRNA occurs at 48 h and is reversible upon withdrawal of IFN-γ from the culture medium. In vitro nuclear run-on transcription assays were performed to directly establish that IFN-γ exerts an early effect at the level of transcription, with maximal transcription rates occurring within 4 h. The difference between the time course of transcription induction and that of mRNA accumulation suggests that the regulation of class I gene expression in this human leukemic cell line also involves posttranscriptional mechanisms. Measurements of cell proliferation rates and cell cycle distribution, as well as the reversibility of the effects of IFN-γ, demonstrate that the selective induction of class I genes in these cells occurs in the absence of differentiation.

Chen, E., *Karr, R. W.*, Frost, J. P., Gonwa, T. A., and Ginder, G. D.

Molecular and Cellular Biology **6**(5):1698-1705, 1986.

Other support: National Institutes of Health.

From the Veterans Administration Medical Center and Department of Internal Medicine, University of Iowa College of Medicine, and Genetics Ph.D. Program, University of Iowa, Iowa City.

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TOWARDS A UNIFIED THEORY OF IMMUNOGLOBULIN STRUCTURE-FUNCTION RELATIONS

The network theory (Jerne 1974) of immune regulation requires selection of binding sites against binding sites. It is difficult to reconcile this axiom with the concept of a series of antibodies in which the binding sites are all composed of interacting residues recessed in a pocket. In this review we address the structural and functional specialization of the antibody molecule from the point of view that there is overlap between idiotypic and paratopic repertoires. This overlap leads to the introduction of multipotentiality at the level of the immunoglobulin itself while still adhering to the clonal selection theory (Burnet 1959). The possible absence of distinction between CDR and idiotope determining regions (IDR) suggests a unified concept of Ig structure which interacts with antigens and idiotopes in the immune network. We call this concept the Variable Surface Recognition Model. Herein we draw upon information about the antigenic structure of proteins, the biological response to these antigenic structures, structural principles to which proteins adhere, anti-idiotypic and synthetic peptide experiments and the structural characteristics of immunoglobulins to justify such a unified concept.

Kieber-Emmons, T. and *Kohler, H.*

Immunological Reviews **90**:29-48, 1986.

Other support: American Cancer Society and the National Institute on Aging.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

IMMUNOGLOBULIN WITH COMPLEMENTARY PARATOPE AND IDIOTOPE

A hybridoma antibody (11E7-1) was isolated from a myeloma fusion with *nu/nu* BALB/c immunized against the T15 idiotype. This IgM antibody exhibited a dual specificity, binding both to PC and to anti-PC antibodies from two idiotype families. Binding to PC and anti-PC antibodies are completely inhibited by PC analogs. Furthermore, the hybridoma antibody binds to itself. Self-binding is also inhibited by PC analogs. From these data, we suggest that 11E7-1 hybridoma antibody has a PC-specific paratope site, and at the same time expresses the internal PC antigen idiotope. The term autobody is proposed to signify its self-binding and potential role in autoimmunity. Autobodies may have a unique role in the network of immune system. Furthermore, it may be a model for designing idiotype vaccines.

Kang, C.-Y. and *Kohler, H.*

Journal of Experimental Medicine **163**:787-796, 1986.

Other support: American Cancer Society and the National Institute on Aging.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

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IDIOTOPE ANTIGENS (Ab2α AND Ab2β) CAN INDUCE IN VITRO B CELL PROLIFERATION AND ANTIBODY PRODUCTION

We previously showed that immunization of various strains of mice with three types of antigen—PC-Hy (nominal antigen), F6-Hy (Ab2α-Hy), and 4C11-Hy (Ab2β-Hy)—induces a differential PC-specific, T15-Id⁺ antibody response. In this report, the in vitro phosphorylcholine (PC)-specific B cell responses induced by these three antigens were studied. A hemocyanin-specific long-term T helper cell line was used to provide help for primary and secondary in vitro T cell-dependent B cell responses. At low doses (0.005 to 0.5 μg/ml) of antigen, a significant increase in the proliferation of PC-OVA-primed BALB/c B cells was observed with Ab2-Hy or PC-Hy conjugate, but not unconjugate, antigens. Similar low doses of antigen could stimulate naive B cells to secrete IgM and stimulate PC-OVA- or 4C11-Hy-primed B cells to secret IgM and IgG1 anti-PC antibodies. The percentage of T15-Id of the PC-specific antibodies produced in the in vitro T-B culture was found to be less dominant than that produced by in vivo immunization, suggesting that certain regulatory mechanisms occur in the in vivo environment that may help to maintain the T15-Id dominance. Taken together, our in vivo and in vitro results indicate that idiotope antigens can function like nominal antigens to induce antigen-specific B cell responses. The mechanisms of thymic-dependent B cell activation induced by idiotope and nominal antigen are similar in that the T-B interaction is MHC-restricted and requires cognate recognition.

Huang, J.-H., Ward, R. E. and *Kohler, H.*

The Journal of Immunology **137**(3):770-776, August 1, 1986.

Other support: National Institute on Aging.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

TUMOR-SPECIFIC IDIOTYPE VACCINES: I. GENERATION AND CHARACTERIZATION OF INTERNAL IMAGE TUMOR ANTIGEN

The concept of idiotype vaccines against tumor-associated antigens (TAA) was tested in the DBA/2 L1210 lymphoma subline, L1210/GZL. Monoclonal antibodies against a TAA that cross-reacts with the envelope glycoprotein gp52 of the mammary tumor virus were used to make hybridoma anti-idiotype antibodies (Ab2). In this report we describe the characterization of monoclonal anti-idiotypic antibodies against the combining site of 11C1 (Ab1), which recognizes a shared determinant of gp52 of mouse mammary tumor virus (MMTV) and the TAA of L1210/GZL. Hybridomas expressing the internal image of gp52 were screened by an idiotype inhibition assay. Mice sensitized with radiated L1210/GZL cells produced specific delayed type hypersensitivity (DTH) against the Ab2 hybridoma. Five Ab2 hybridomas were selected and were used to immunize DBA/2 mice. Such immunized animals showed specific DTH reaction against a challenge with the L1210/GZL tumor cells. Similar results were obtained in mice immunized with purified Ab2. Fluorescence-activated cell sorter analysis demonstrated that fluorescence staining of L1210/GZL cells by 11C1 can be completely inhibited with preabsorption on Ab2 hybridoma cells. Mice immunized with 2F10 and 3A4 coupled to keyhole limpet hemocyanin (KLH) contained antibodies binding to MMTV. But only in mice immunized with 2F10-KLH was significant inhibition of L1210/GZL tumor growth observed. Collectively, these results indicate that certain

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

anti-idiotypic antibodies can mimic the MMTV gp52 antigen, as well as the gp52-like epitope expressed on the L1210 GZL tumor cells. These properties of anti-idiotypic antibodies mimicking TAA could be exploited for making idiotype vaccines against tumors.

Raychaudhuri, S., Saeki, Y., Fuji, H., and *Kohler, H.*

The Journal of Immunology **137**(5):1743-1749, 1986.

Other support: American Cancer Society and National Cancer Institute.

From the Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY.

CIGARETTE SMOKING AND BRONCHOALVEOLAR T-CELL POPULATIONS IN SARCOIDOSIS

Pulmonary physicians must often deal with patients, including patients with sarcoidosis, who smoke cigarettes. Since changes in local pulmonary immune function have been associated with both sarcoidosis and cigarette smoking, it is important to distinguish which of these immunological changes in the lungs are due to the disease, which are due to cigarette smoking, and which, perhaps, are due to both.

Abnormally large numbers of helper thymus-derived (T) lymphocytes are found in fluids recovered by bronchoalveolar lavage (BAL) from patients with sarcoidosis. By contrast, normal numbers of lymphocytes are found in BAL fluids from normal cigarette smokers, and lower than normal percentages of the cells in these fluids are lymphocytes. The effects of smoking on T lymphocyte subpopulations in normal cigarette smokers and on those in patients with sarcoidosis, however, have not been thoroughly characterized.

The purposes of this study, therefore, were 1) to determine the effects of smoking on T lymphocyte subpopulations in BAL fluids from healthy normal volunteers (''normals'') and on those in BAL fluids from patients with sarcoidosis (''sarcoids''), and 2) to determine whether comparisons of the T lymphocyte subpopulations of normals and those of sarcoids revealed any effects of cigarette smoking.

Lawrence, E. C. et al.

Annals of the New York Academy of Sciences **465**:657-664, June 6, 1986.

Other support: National Institutes of Health and the General Clinical Research Center.

From the Department of Medicine, Baylor College of Medicine, Houston.

IMMUNOCHEMICAL AND PHARMACOLOGICAL DISTINCTIONS BETWEEN CURAREMIMETIC NEUROTOXIN BINDING SITES OF CENTRAL AUTONOMIC, AND PERIPHERAL ORIGIN

Comparative pharmacological and immunochemical studies were conducted on α-bungarotoxin binding sites from rat brain or muscle, *Torpedo* electric tissue, or the TE671 or PC12 clonal cell lines. Characteristic distinctions were observed in the pharmacological profile of drugs competing for toxin binding to different tissues. Differences also were found in the proportion of toxin binding sites (membrane-bound

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

or detergent-solubilized) that are immunologically reactive with either monoclonal antibodies directed against nicotinic acetylcholine receptors from the electric organ of *Torpedo* or polyclonal antisera raised against nicotinic receptors from the electric organ of *Electrophorus*. These results suggest that toxin binding sites are structurally heterogeneous. Structural heterogeneity of nicotinic acetylcholine receptors, neurotoxin binding sites, or both, may contribute to the manifestation of nicotinic receptor functional heterogeneity and may explain the apparent discrepancy at some sites between toxin binding activity and toxin functional potency.

Lukas, R. J.

Proceedings of the National Academy of Sciences, USA **83**:5741-5745, August 1986.

Other support: National Institutes of Health, the Epilepsy Foundation of America, Epi-Hab Phoenix, and the Men's and Women's Boards of the Barrow Neruological Foundation.

From the Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ.

IN VITRO T CELL-MEDIATED KILLING OF *PSEUDOMONAS AERUGINOSA*: II. THE ROLE OF MACROPHAGES AND T CELL KILLING

T lymphocytes from immune mice can adoptively transfer protection against infection with the extracellular Gram-negative bacterium *Pseudomonas aeruginosa* to nonimmune recipients, and in vitro, immune T cells are able to kill these bacteria. Earlier studies indicated that this killing is mediated by a bactericidal lymphokine. Those studies also showed that macrophages enhance this in vitro T cell killing but do not directly participate in the bacterial killing, nor do macrophages function to present antigen to T cells. The current studies demonstrate that the ability of macrophages to enhance T cell killing can be replaced by macrophage culture supernatants or by purified recombinant interleukin 1 (IL 1). In addition, the macrophage supernatant-induced enhancement can also be blocked by antibody to purified IL 1. These studies also demonstrate that the T cell subset that serves as the final effector cell in the killing process is the Lyt-1$^-$, 2,3$^+$, I-J$^+$ phenotype.

Markham, R. B., et al.

The Journal of Immunology **134**(6):4112-4117, June 1985.

Other support: National Institutes of Health and the National Science Foundation.

From the Departments of Medicine and of Microbiology and Immunology, Washington University School of Medicine, and the Jewish Hospital at Washington University Medical Center, St. Louis.

IN VITRO T CELL-MEDIATED KILLING OF *PSEUDOMONAS AERUGINOSA* III. THE ROLE OF SUPPRESSOR T CELLS IN NONRESPONDER MICE

T lymphocytes from immune BALB/c mice can adoptively transfer protection against infection with the extracellular Gram-negative bacterium *Pseudomonas aeruginosa* to nonimmune recipients, and *in vitro*, immune T cells are able to kill these bacteria. Earlier studies indicated that this killing is mediated by a bactericidal lympho-

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

kine. The current studies demonstrate that T cells from immunized CB.20 mice, a strain congenic with BALB/c, fail to kill *Pseudomonas aeruginosa* in vitro. This nonresponsiveness is attributable to the activity of suppressor T cells of the Lyt-1$^+$, 2,3$^+$, I-J$^+$ phenotype. CB.20 mice are known to differ from BALB/c mice only at a single locus, which includes the Igh-1 allotype C_μ genes. These results suggest a critical role for this locus or closely linked genes in the control of T cell killing of this extracellular bacterium.

Powderly, W. G., Pier, G. B. and *Markham, R. B.*

The Journal of Immunology **136**(1):299-303, January 1, 1986.

Other support: National Institutes of Health.

From the Departments of Medicine and of Microbiology and Immunology, Washington University School of Medicine, and the Jewish Hospital at Washington University Medical Center, St. Louis.

T LYMPHOCYTE-MEDIATED PROTECTION AGAINST *PSEUDOMONAS AERUGINOSA* INFECTION IN GRANULOCYTOPENIC MICE

BALB/c mice immunized with *Pseudomonas aeruginosa* immunotype 1 polysaccharide develop protective T cell immunity to bacterial challenge. *In vitro*, T cells from immunized mice kill *P. aeruginosa* by production of a bactericidal lymphokine. The present study demonstrates that adoptive transfer of T cells from immunized BALB/c mice to granulocytopenic mice resulted in 97% survival on challenge with *P. aeruginosa*, compared with 17% survival with adoptive transfer of T cells from nonimmune BALB/c mice. This protection is specifically elicited by reexposure to the original immunizing antigen; adoptive recipients cannot withstand challenge with immunotype 3 *P. aeruginosa*. However, the adoptive recipients do survive simultaneous infection with both *P. aeruginosa* immunotypes 1 and 3. Adoptive transfer of T cells from the congenic CB.20 mice, which are unable to kill *P. aeruginosa* in vitro, provides only 20% protection to granulocytopenic mice. These studies indicate that transfer of specific immune T lymphocytes can significantly enhance the resistance to *P. aeruginosa* infection in granulocytopenic mice.

Powderly, W. G., Pier, G. B. and *Markham, R. B.*

Journal of Clinical Investigation **78**:375-380, August 1986.

Other support: National Institutes of Health.

From the Departments of Medicine and of Microbiology and Immunology, Washington University School of Medicine, and the Jewish Hospital at Washington University Medical Center, St. Louis.

IN VITRO T CELL-MEDIATED KILLING OF *PSEUDOMONAS AERUGINOSA*: IV. NONRESPONSIVENESS IN POLYSACCHARIDE-IMMUNIZED BALB/c MICE IS ATTRIBUTABLE TO VINBLASTINE-SENSITIVE SUPPRESSOR T CELLS

We reported previously that BALB/c mice immunized with a polysaccharide (PS) antigen isolated from immunotype 1 *Pseudomonas aeruginosa* and vinblastine

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

sulfate develop T cell-mediated protective immunity, despite their failure to produce specific antibody. *In vitro*, Lyt-1$^-$,2$^-$, I-J$^+$ T cells from vinblastine- and PS-immunized mice kill *P. aeruginosa* by secretion of a bactericidal lymphokine. BALB/c mice immunized with PS alone generate neither protective antibodies nor a protective T cell response. The current studies indicate that T cells from mice immunized with PS alone significantly suppress the bactericidal activity of T cells from mice immunized with vinblastine and PS. The suppressor T cells are of the same Lyt-1$^-$,2$^-$, I-J$^+$ phenotype as the bactericidal T cells. Suppression is mediated by a soluble product of these suppressor T cells which both inhibits T cell proliferation and interferes with the production or release of the bactericidal lymphokine. Cyclophosphamide, used in other systems to remove suppressor T cells, fails to enhance bacterial killing and does not inhibit suppressor cell activity. These studies indicate that immunization with PS elicits responses in two functionally distinct subgroups of Lyt-1$^-$,2$^-$, I-J$^+$ T cells, and that these cells are distinguishable by their sensitivity to vinblastine sulfate.

Powderly, W. G., Pier, G. B. and *Markham, R. B.*

The Journal of Immunology **137**(6):2025-2030, September 15, 1986.

Other support: National Institutes of Health.

From the Departments of Medicine and of Microbiology and Immunology, Washington University School of Medicine, and the Jewish Hospital at Washington University Medical Center, St. Louis.

MUTUAL RELATIONSHIP AMONG CYTOSOLIC pH, Na^+ AND Ca^{++} IONS IN THE DEGRANULATION OF RAT LEUKEMIC BASOPHILS

Reagents which affect the cytosolic concentrations of protons and sodium ions markedly affect the degranulation process of mast cells. The proton–sodium exchanging ionophore, monensin, is found to cause noncytolytic dose dependent serotonin release from the rat leukemic basophils (line RBL-2H3). Its half maximal dose of ca. 2 μM leads to secretion of ca. 20% of these cells' serotonin content. Monensin induced serotonin secretion increases with external pH and decreases upon lowering external sodium ion concentrations, yet is independent on external calcium. Monitoring cytosolic pH and free Ca^{++} concentrations with BCECF and quin2, respectively, shows that a rise in pH$_i$ and [Ca^{++}]$_i$ is caused by the ionophore. Amiloride, the blocker of cellular Na^+/K^+ antiporter, is found to be an effective inhibitor of antigen or monensin induced serotonin release. However, it does not by itself cause secretion. In contrast, ouabain, which inhibits the cellular Na^+/K^+ ATPase, does induce secretion. Cellular levels of pH, Na^+ and Ca^{++} ions are evidently linked and involve a manifold of activities. Though exchanging protons for sodium seems to be effective in causing mediator release, the present results do not provide sufficient support for proton/sodium ions having a second messenger role in the immunologically induced mediator release.

Sussman, Y., Reck, B. and *Pecht, I.*

Immunology Letters **13**:215-219, 1986.

From the Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel.

Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

IMMUNOLOGIC PROPERTIES OF ENDOTHELIAL CELLS

Endothelial seeding of vascular grafts has been shown to reduce thrombogenicity, improve patency rates, and improve graft incorporation. However, endothelial cells possess the major histocompatibility antigens, and antibodies directed against endothelium have been associated with graft rejection. At present, the best approach seems to be to avoid anti-donor immunologic reactivity by seeding autologous endothelial cells onto grafts prior to implantation. The surface of endothelial cells normally is actively antithrombogenic by virtue of the cells' abilities to synthesize and release PGI_2, and because of a surface ADPase. However, endothelium that is injured by viral infection, bacterial endotoxin, white blood cell lysates, or antibodies to endothelial surface antigens in the presence of complement, can bind immune complexes and can support complement-linked and procoagulant activities leading to vascular occlusion and damage. Nevertheless, the advantages of an endothelial lining are such that efforts should be made to understand better the conditions that may lead to endothelial dysfunction.

Ryan, U. S.

Asaio 8(2):58-64, April-June 1985.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

ISOLATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES REACTIVE WITH ENDOTHELIAL CELLS

Monoclonal antibodies were generated to antigens on cultured human umbilical vein endothelial cells. Spleen cells from BALB/c mice, immunized with low passage cultures of human umbilical vein endothelial cells, were fused with the non-secretory myeloma line, P3 × 63Ag 8.653. Hybridoma supernatants were screened for the desired immunological reactivity using ELISA binding assays. Hybridomas secreting antibodies reacting with the immunizing endothelial cells, but not with peripheral blood mononuclear cells, were cloned by limiting dilution and three stable clones were chosen for study. Further testing by ELISA revealed that each antibody displayed a unique pattern of reactivity. One antibody, 14E5, reacted with the macrophage-like cell line DHL-2, cultured macrophages derived from peripheral blood monocytes, and macrophages derived from malignant effusions. The antibody failed to react with fibroblasts or bovine endothelial cells. The second antibody, 12C6, reacted with human and primate fibroblasts and endothelial cells derived from bovine arteries, but not with mature macrophages. The third clone, 10B9, reacted only with immunizing endothelial cells and the immature-macrophage line U-937. All three antibodies failed to react with long-term human B or T lymphoblastoid cell lines, leukemic cell lines, or murine macrophage lines. None of the antibodies reacted with a battery of human epithelial derived cell lines or primary cultures of human epithelial cells. Indirect immunofluorescence assays revealed that the antigens were expressed on the cell surface. These antibodies should prove useful as differentiation markers of human endothelial cells and in studies of endothelial cell function.

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

Hamburger, A. W., Reid, Y. A., *Ryan, U.* and *Cines, D. B.*

Tissue & Cell **17**(4):451-459, 1985.

Other support: National Institutes of Allergy and Infectious Disease and a Research Career Development Award.

From the Cell Culture Department, American Type Culture Collection, Rockville, MD.

REGULATION OF T CELL AUTOCRINE GROWTH

During the course of investigating the regulation of IL-2-dependent T cell proliferation, we found that the subset of human T cells expressing the T4 surface glycoprotein become refractory to IL-2 growth promotion earlier than $T8^+$ cells. Since $T4^+$ cells proliferate in an autocrine fashion to endogenous IL-2, whereas most $T8^+$ cells respond in a paracrine fashion to IL-2 derived from $T4^+$ cells, we thought it likely that a unique mechanism was operative to restrict $T4^+$ cell IL-2-dependent autocrine proliferation. Moreover, we anticipated that the $T4^+$ cell IL-2-refractory state related either to suppression by $T8^+$ cells, or to expression of $T4^+$ cell IL-2-R. However, several experimental approaches did not support either of these mechanisms as being responsible for the loss of $T4^+$ cell IL-2 responsiveness. Isolated $T4^+$ cells ceased to respond to IL-2 well before $T8^+$ cells, and before the disappearance of adequate levels of IL-2-R. Moreover, a detailed comparison of IL-2-R expression by $T4^+$ vs. $T8^+$ cells revealed no differences in the number, affinity, rate of expression, or functional activity of high-affinity IL-2-R expressed by the two subsets. Accordingly, $T4^+$ cell autocrine IL-2 responsiveness is restricted by a mechanism that is independent of IL-2-R, and which ultimately results in cessation of both $T4^+$ and $T8^+$ cell IL-2-dependent clonal expansion.

Gullberg, M. and *Smith, K. A.*

Journal of Experimental Medicine **163**:270-284, February 1986.

Other support: National Cancer Institute and the Eli Lilly Corporation.

From the Department of Medicine, Dartmouth Medical School, Hanover, NH.

ANTIBODIES SPECIFIC FOR THE Mac-1, LFA-1, p150,95 GLYCOPROTEINS OR THEIR FAMILY, OR FOR OTHER GRANULOCYTE PROTEINS

In this report from the 2nd International Workshop on Human Leukocyte Differentiation Antigens, the authors note that a family of functionally important leukocyte surface glycoproteins which share a common β subunit of M_r = 95,000 has recently been defined in humans and mice. These glycoproteins, the lymphocyte function-associated 1 (LFA-1), macrophage 1 (Mac-1), and p150,95 molecules, each contain a different α subunit noncovalently associated with the common β subunit in an α,β structure. Monoclonal antibodies specific for the LFA-1 and Mac-1 molecules have allowed definition of their cell distribution and functions.

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

The LFA-1 molecule is expressed on B and T lymphocytes, NK cells, monocytes, and granulocytes. Monoclonal antibodies to LFA-1 block cytolytic T lymphocyte-mediated killing, natural killing and T helper cell responses. The Mac-1 molecule, identical to OKM1 and Mol1, is expressed on granulocytes, monocytes, and natural killer cells, and is absent on lymphocytes. The third member of the family, p150,95, named after the $M_r \times 10^3$ of its subunits, has been defined biochemically after isolation from myeloid cells with anti-β mAb.

In this study, the 118 mAbs submitted in the myeloid panel of the Second International Conference on Human Leukocyte Differentiation Antigens were tested for reactivity with members of the Mac-1, LFA-1, p150-95 family. It was of interest to compare mAbs from different laboratories, which have been the subject of several publications. Furthermore, the following questions were addressed: (1) Which mAbs reacted with specific members of the family and which cross-reacted with all three members? did any show unusual specificities, such as cross-reaction between only two members of the family? (2) Could mAbs specific for the p150,95 molecule be identified in the panel? (3) Which members of the family were up-regulated on myeloid cell surfaces by chemo-attractants? (4) Which mAbs in the myeloid panel were negative on Mac-1, LFA-1-deficient patients? How specific was the deficiency to the Mac-1, LFA-1 deficient patients? How specific was the deficiency to the Mac-1 LFA-1, p150-95 glycoprotein family?

In the course of these studies, information was also obtained on molecules distinct from Mac-1, LFA-1, and p150,95. This is presented as an appendix.

Springer, T. A. and Anderson, D. C.

In: Reinherz, E., Haynes, B., Nadler, L., Bernstein I. (eds.): *Leukocyte Typing II: Volume 3, Human Myeloid and Hematopoietic Cells*, New York: Springer-Verlag, 1986, Chap. 4, pp. 55-68.

Other support: National Institutes of Health.

From the Harvard Medical School, Boston.

MECHANISMS OF TUMOR CELL CAPTURE BY ACTIVATED MACROPHAGES: EVIDENCE FOR INVOLVEMENT OF LYMPHOCYTE FUNCTION-ASSOCIATED (LFA)-1 ANTIGEN

The lymphocyte function-associated (LFA)-1 molecule is expressed on certain populations of macrophages that have an augmented capacity to capture tumor cells. Accordingly, we analyzed the role of LFA-1 in the establishment of such cell-cell interactions. F(ab')$_2$ fragments of the M17/4, anti-LFA-1 monoclonal antibody (MAb) inhibited the interaction between activated macrophages and tumor cells by up to 80% in a dose-dependent manner. The anti-LFA-1 MAb reduced (between 55 to 79%) the number pf P815, LSTRA, or EL-4 tumor cells bound to trypsin-sensitive structures on bacillus Calmette Guerin activated macrophages. The inhibition appeared selective, because a F(ab')$_2$ fragment of anti-MAC-1 did not inhibit such binding. Inhibition of tumor cell capture could be observed as soon as 15 min after the onset of the cell-cell interaction between activated macrophages and tumor cells. Optimal inhibition occurred when both tumor targets and macrophages were precoated with the MAb.

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

Although P815, LSTRA, EL-4 and BW5147 tumor cells all expressed LFA-1, only the first three but not BW5147 cells were bound by activated macrophages. Furthermore, endotoxin-pulsed macrophages elicited by thioglycollate broth expressed the LFA-1 antigen but did not exhibit selective tumor cell capture. Finally, anti-LFA-1 inhibited the development of weak into strong binding. Taken together, the results suggest that LFA-1 molecules can participate in the interaction between activated macrophages and neoplastic cells.

Strassmann, G., *Springer, T. A.*, Sommers, S. D., and Adams, D. O.

The Journal of Immunology 136 (**11**): 4328-4333, June 1, 1986.

Other support: U. S. Public Health Service and R. J. Reynolds Industries, Inc.

From the Deparment of Microbiology-Immunology, Duke University Medical Center, Durham, NC.

VII. *Metabolic Studies*

THE MECHANISM OF ACTION OF LYMPHOKINES. IX. THE ENZYMATIC BASIS OF HYDROGEN PEROXIDE PRODUCTION BY LYMPHOKINE-ACTIVATED MACROPHAGES

The purpose of this study was to elucidate the biochemical basis of the enhanced hydrogen peroxide (H_2O_2) production by guinea pig peritoneal macrophages (MP) cultured in lymphokine (LK)-containing medium. The markedly augmented H_2O_2 generation by these cells, demonstrable by the horseradish peroxidase (HRP)-catalyzed oxidation of phenol red, is distinguished by its lack of dependence on a second stimulus. We demonstrate that H_2O_2 production is truly spontaneous and is not caused by a stimulant present among the H_2O_2 assay reagents. The principal candidate for such a role was HRP type II (a mixture of five isoenzymes) that was reported to be capable of eliciting an oxidative burst in MP. Four distinct HRP isoenzymes that were found incapable of provoking an oxidative response were nevertheless adequate for demonstrating H_2O_2 production by LK-activated MP. Blocking the MP receptor for mannose by the addition of mannan to the assay system resulted in enhanced detection of H_2O_2 by low concentrations of HRP type II and by three out of four HRP isoenzymes. Treatment of MP with LK-containing medium for 72 hr did not result in a significant change in the activity of cellular superoxide dismutase (SOD) compared with MP cultured for the same length of time in control medium.

By using the specific inhibitor of copper, zinc-containing SOD, sodium diethyldithiocarbamate (DDC), and the universal SOD inhibitor, sodium nitroprusside, we found that the predominant enzyme in guinea pig peritoneal MP is probably manga-

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

nese-containing SOD. Incubation of LK-activated MP with nitroprusside resulted in almost total inhibition of H_2O_2 production and a simultaneous switch to superoxide (O_2^-) liberation. Similar exposure to DDC had no effect. These data indicate that H_2O_2 produced by LK-activated MP is derived exclusively by enzymatic dismutation of O_2^- mediated by a manganese-containing SOD. The increase in spontaneous H_2O_2 production induced by LK is therefore secondary to augmented O_2^- production that occurs at a cellular location where O_2^- is accessible to SOD.

The enzymatic basis of the enhanced oxygen radical production was investigated by determining the kinetic parameters of the O_2^--forming NADPH oxidase of resting LK-treated MP in a cellfree system in which O_2^- production was induced by sodium dodecyl sulfate. The K_m for NADPH and the V_{max} of the enzyme of LK-treated MP were not different from those of the enzyme of MP incubated in control medium. We conclude that LK treatment of MP does not modulate the NADPH oxidase itself but, most likely, a process related to activation of the enzyme.

Freund, M. and *Pick, E.*

The Journal of Immunology **137**(4):1312-1318, August 15, 1986.

Other support: A. Plesch Research Foundation.

From the Laboratory of Immunopharmacology, Department of Human Microbiology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

THE MACROPHAGE-MEDIATED REGULATION OF HEPATOCYTE SYNTHESIS OF ANTITHROMBIN III AND α_1-PROTEINASE INHIBITOR

Antithrombin III (ATIII) is an anticoagulant protein which binds and inactivates thrombin and other serine proteinases. Little is known about regulation of its synthesis. We confirm that ATIII is synthesized by isolated rat hepatocytes and that its synthesis is not altered by direct feedback of its complexes with proteinases. Neither is hepatocyte synthesis of ATIII altered by supernatants from macrophages cultured in the presence of ATIII-proteinase complexes. However, culture of macrophages with fibrinogen fragment D results in production of a factor(s) in the macrophage supernatants which stimulates hepatic fibrinogen synthesis, as previously described, and also stimulates the synthesis of ATIII and α_1-proteinase inhibitor. Synthesis of albumin and rat α_2-macroglobulin is not altered. Culture of macrophages in the presence of bacterial endotoxin also results in release of a factor(s) into the medium which stimulates the same changes in hepatocyte protein synthesis. These results show for the first time a mechanism by which synthesis of ATIII can be regulated during coagulation and fibrinolysis.

Hoffman, M., Fuchs, H. E., and *Pizzo, S. V.*

Thrombosis Research **41**:707-715, 1986.

Other support: National Institutes of Health.

From the Departments of Pathology, Biochemistry and Surgery, Duke University Medical Center, Durham, NC.

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VIII. *Epidemiology*

GENETIC-ENVIRONMENTAL INTERACTIONS IN CHRONIC AIRWAYS OBSTRUCTION

To examine smoking and genetic factors in relation to airways obstruction, cross sectional data were analyzed on 1,787 white non-patient adult participants in a genetic-epidemiological study of airways obstruction (AO), defined as one-second forced expiratory volume (FEV1) less than 68% of forced vital capacity (FVC). Interaction was examined between smoking and each of four factors previously found to be related to AO: alpha-1 antitrypsin (PiZ allele), ABO blood groups (A antigen), ABH non-secretor status, and first degree relationship to a chronic obstructive pulmonary disease or lung cancer patient. Multiple linear regression was used to test for interaction and adjust mean FEV1 (as a per cent of FVC) and prevalence of AO for age, sex, socioeconomic status, coffee and alcohol intake. Statistical interaction was observed between smoking (measured in pack-years) and two genetic factors (presence of blood A antigen and the family history). At higher pack-years levels, those individuals with the A antigen or the family history, but especially those with both factors, had a much lower mean FEV1/FVC% and a much higher prevalence of AO than expected based on a simple additive model. On the other hand, there was no interaction between smoking and PiZ allele, or smoking and ABH secretory status. The findings suggest a possible interaction between cigarette smoke and the airways of individuals with blood group A antigen and familial lung disease. The findings also emphasize the role of genetic-environmental interactions in chronic diseases of multifactorial etiology.

Khoury, M. J. *et al.* (*Cohen, B. H.*)

International Journal of Epidemiology **15**(1):65-72, 1986.

Other support: Lebanese National Council for Scientific Research.

From the Department of Epidemiology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

MILK DRINKING AND POSSIBLE PROTECTION OF THE RESPIRATORY EPITHELIUM

In a Hopkins investigation, detailed interviews as well as spirometry were obtained on 2,539 non-patient adult participants. The interviews included questions regarding smoking habits, family history, socioeconomic status, respiratory symptoms, certain dietary factors, and beverage consumption. For the analyses of risk factors, all patients were excluded from the original study population, which consisted not only of several groups of patients along with their relatives, but also neighborhood controls, teachers and other groups. Thus, only those subjects over 20 years of age who were not ascertained on the basis of their own health status were considered. Chronic bronchitis (CB) was identified by the report of cough and phlegm production for three or more months per year for two consecutive years. Results of this study showed that milk drinking was inversely associated with CB in this genetic-epidemiologic study of chronic lung disease risk factors at The Johns Hopkins Medical Institutions. This finding is of particular interest in view of the well-documented association of vitamin A

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

deficiency with inflammatory epithelial changes as well as the recent reports suggesting a role of vitamin A in decreased risk of neoplastic changes in the lung.

Tockman, M. S., Khoury, M. J. and *Cohen, B. H.*

Journal of Chronic Diseases **39**(3):207-209, 1986.

Other support: National Heart, Lung and Blood Institute.

From the Department of Environmental Health Sciences and Department of Epidemiology, The Johns Hopkins University, Baltimore, MD.

CIGARETTE SMOKING CHARACTERISTICS OF A SMALL POPULATION OF VOLUNTEER BLOOD DONORS

As part of a three-year study of the effects of cigarette smoking on blood components, demographic data was obtained from healthy volunteer smokers responding to bulletin board notices of solicitation. In addition to age and sex, the type (brand) and number of cigarettes smoked were recorded for each smoker on forms developed to assure collection of data in a uniform and reliable manner. Analysis of the date at completion of the study period revealed that a majority of smokers use filtered versus non-filtered cigarettes (85 vs. 4; $P \leq .05$, Z-test); of the filtered variety, a majority were non-menthol versus menthol (60 vs. 29; $P \leq .05$, Z-test). The 1984 Federal Trade Commission values for each brand's CO, tar and nicotine per cigarette were multiplied by each corresponding donor's reported number of cigarettes per day to estimate total (maximum) CO, nicotine and tar exposure per day; average daily exposures for all smokers were 23 ± 2.43 (SEM) cigarettes per day, 275 ± 20.2 mg CO; 270 ± 21.2 mg tar and 18 ± 1.3 mg nicotine. Males and females were not significantly different. Smokers over age 35 smoked a larger number of cigarettes per day (26.8 ± 2.1 vs. 20.5 ± 1.5; $P \leq .02$, t-test) and thus had higher smoke component exposures. There was no age difference in non-filter cigarette use. These data showing the same use and types of cigarettes for male and female smokers and increases in the number of cigarettes per day in both groups with increased age contrast with data for larger populations less than 15 years ago.

Beyers, B. J., Bowen, R. J., Panus, P. and *Longenecker, G. L.*

The Journal of Research Communications in Substance Abuse **7**(1 & 2):49-57, 1986.

From the Department of Pharmacology, College of Medicine, University of South Alabama, Mobile.

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Source: https://www.industrydocuments.ucsf.edu/docs/kqxk0000

Active Projects

Following is a list of the principal investigators, or institutions, whose projects are underway or were activated in the period since the previous Report, together with the respective project titles. Completed projects are listed in a later section.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
ROBERT H. ABELES, PH.D. *Professor of Biochemistry*, Brandeis University, Waltham, MA.	Development of elastase inhibitors
LEO G. ABOOD, PH.D. *Professor of Brain Research and Biochemistry*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Nicotine transfer-disposition in liver cells
DOLPH O. ADAMS, M.D., PH.D. *Professor of Pathology*, Duke University Medical Center, Durham, NC.	Role and regulation of protein phosphorylation during macrophage activation
IAN Y. R. ADAMSON, PH.D. *Professor of Pathology*, University of Manitoba, Winnipeg, Manitoba, Canada.	Cell interactions at the air blood barrier
BURT ADELMAN, M.D. *Assistant Professor of Medicine*, Medical College of Virginia, Richmond.	Effect of fibrinolytic activation on platelet function
KENNETH B. ADLER, PH.D. *Assistant Professor of Pathology*, University of Vermont College of Medicine, Burlington.	Airway mucin secretion: effects of products from bacteria associated with chronic bronchitis
JOHN J. ALBERS, PH.D. *Research Associate Professor of Medicine*, University of Washington School of Medicine, Seattle.	High density lipoprotein quantitation
HARRY N. ANTONIADES, PH.D. *Professor of Biochemistry*, Harvard University School of Public Health, Boston.	Biosynthesis and processing of PDGF-like polypeptides in human malignant cells in culture
IRIT AVIRAM, PH.D. *Department of Biochemistry*, The Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.	Isolation, properties and physiological function of neutrophil cytochrome *b*
BERNARD M. BABIOR, M.D., PH.D. *Head, Division of Biochemistry*, Scripps Clinic and Research Foundation, La Jolla, CA.	Studies on the mechanism of activation of the respiratory burst in neutrophils
LAURIE BARCLAY, M.D. *Clinical Director*, The Burke Rehabilitation Center, White Plains, NY.	Tobacco use in Alzheimer's disease
MICHAEL BARANY, M.D., PH.D. *Professor of Biological Chemistry*, University of Illinois, Chicago.	Effect of drugs on membranes of live tissues

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
DAVID W. BARNES, PH.D. *Associate Professor of Biochemistry Biophysics*, Oregon State University, Corvallis.	Identification of oncogenes involved in human lung carcinoma
JOEL S. BENNETT, M.D. *Associate Professor of Medicine*, Hospital of the University of Pennsylvania, Philadelphia	Characterization of the platelet fibrinogen receptor
RICHARD J. BING, M.D. *Professor of Medicine (emeritus)*, University of Southern California School of Medicine, Los Angeles; *Visiting Associate*, California Institute of Technology; *Director of Experimental Cardiology and Scientific Development*, Huntington Medical Research Institutes, Pasadena, CA.	Coronary spasm, cerebral microcirculation
THOMAS R. BROKER, PH.D. *Associate Professor of Biochemistry*, University of Rochester School of Medicine, Rochester, NY.	Cellular transformation by papilloma virus recombinants
DOROTHY L. BUCHHAGEN, PH.D. *Assistant Professor*, State University of New York, Downstate Medical Center, Brooklyn, NY.	Oncogene expression in fetal mouse lung
VINCENZO BUONASSISI, M.D. *Senior Scientist and Deputy Director*, W. Alton Jones Cell Science Center, Inc., Lake Placid, NY.	Heparan sulfate proteoglycans and blood homeostatic mechanisms
JOHN W. BURCH, M.D. *Associate Medical Director*, American Red Cross, Rochester Division, Rochester, NY.	Control of arachidonic acid oxygenation in human platelets
DAVID L. BUSBEE, PH.D. *Professor of Toxicology*, Texas A&M University College of Veterinary Medicine, College Station.	Polynuclear aromatic hydrocarbon transport by serum lipoproteins
EDWARD J. CAMPBELL, M.D. *Assistant Professor of Medicine*, Washington University School of Medicine, St. Louis, MO.	Modulators of inflammatory cell proteolytic activity
DENNIS A. CARSON, M.D. *Associate Member*, Scripps Clinic and Research Foundation, La Jolla, CA.	Mechanism of immune dysfunction after oxidant exposure
DONNA CHAPRONIERE, B.SC., PH.D. Strangeways Research Laboratory, Cambridge, England.	Control of proliferation of cells from the adult human prostate
LAN BO CHEN, PH.D. *Associate Professor of Pathology*, Dana-Farber Cancer Institute, Boston	Studies on human oat cell carcinomas
YUAN-TSONG CHEN, M.D., PH.D. *Assistant Professor of Pediatrics*, Duke University Medical Center, Durham, NC.	Recombinant DNA approaches to assess risk for lung cancer

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
MORDECHAI CHEVION, PH.D. *Chairman*, Institute of Biochemistry, The Hebrew University of Jerusalem, Jerusalem, Israel	The effects of Vitamin C and transition metals on coagulation processes
WILLIAM M. CHILIAN, PH.D. *Assistant Research Scientist*, Cardiovascular Center, University of Iowa College of Medicine, Iowa City.	Pathophysiology of the coronary microcirculation
DOUGLAS BROCK CINES, M.D. *Professor of Medicine*, Hospital of the University of Pennsylvania, Philadelphia.	Immune injury of human endothelial cells
CURT I. CIVIN, M.D. *Assistant Professor of Oncology & Pediatrics*, The Johns Hopkins Oncology Center, Baltimore, MD.	Biochemistry and function of human granulopoietic antigens
ROBERT A. CLARK, M.D. *Professor of Medicine*, University of Iowa, Iowa City.	Biosynthesis of human neutrophil elastase
GARY A. CLAWSON, M.D., PH.D. *Assistant Professor*, University of California, San Francisco.	Nuclear NTPase and selective RNA splicing / transport
BRIAN L. CLEVINGER, PH.D. *Assistant Professor of Biomedical Science*, Washington University of Dental Medicine, St. Louis, MO.	Role of J segment in V segment expression
CHARLES G. COCHRANE, M.D. *Member, Department of Immunopathology*, Scripps Clinic and Research Foundation, La Jolla, CA.	Mediation systems in inflammatory lung disease
BERNICE H. COHEN, PH.D. *Professor of Epidemiology*, The Johns Hopkins University, Baltimore, MD.	Airways obstruction and smoking in black and white adults
ROBERT W. COLMAN, M.D. *Professor of Medicine*, Temple University School of Medicine, Philadelphia.	Initiation of plasma coagulation and kinin forming systems in man
ROBERT L. CONHAIM, PH.D. *Associate Scientist*, University of Wisconsin, Madison.	Routes of alveolar flooding and clearance
GERALD R. CRABTREE, M.D. *Associate Professor of Pathology*, Stanford University, Stanford, CA.	Retroviral insertion and activation of the IL-2 gene
EVA BROWN CRAMER, PH.D. *Associate Professor of Anatomy and Cell Biology*, Downstate Medical Center, Brooklyn, NY.	Studies of inflammation using an *in vitro* model
CARL E. CRUETZ, PH.D. *Assistant Professor of Pharmacology*, University of Virginia School of Medicine, Charlottesville.	Role of protein phosphorylation in nicotine-induced catecholamine release

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PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
GIDON CZAPSKI, M.Sc., Ph.D. *Professor of Physical Chemistry*, The Hebrew University of Jerusalem, Jerusalem, Israel	Role of metal ions on superoxide and Vitamin C toxicity in biological systems
IVAN DAMJANOV, M.D., Ph.D. *Professor of Pathology*, Jefferson Medical Center, Thomas Jefferson University, Philadelphia	Developmentally pluripotent human lung cancer stem cells
ALBERT B. DEISSEROTH, M.D., Ph.D. *Professor of Medicine*, Veterans Administration Medical Center, San Francisco	Study of altered alpha globin genes in leukemia and solid tumors
MARTIN E. DORF, Ph.D. *Professor of Pathology*, Harvard Medical School, Boston	Macrophage-like cells involved in immune suppression
PETER H. DUESBERG, Ph.D. *Professor of Molecular Biology*, University of California, Berkeley	Transforming genes of two acute leukemia viruses
REUBEN EISENSTEIN, M.D. *Professor of Pathology*, Mount Sinai Medical Center, Milwaukee, WI	Heparin-binding proteins and endothelial cells
ALVAN R. FEINSTEIN, M.D. *Professor of Medicine and Epidemiology*, Yale University School of Medicine, New Haven, CT	Smoking, detection bias and primary lung cancer
PAUL B. FISHER, Ph.D. *Senior Research Associate*, Department of Microbiology, Columbia University College of Physicians & Surgeons, New York	Chemical-viral interactions in cell transformation
JUDITH ANN FOSTER, Ph.D. *Professor and Chairperson, Department of Biology*, Syracuse University, Syracuse, NY	Involvement of elastin fibers in lung disease
RICHARD B. FOX, M.D. *Assistant Professor of Pediatrics*, Children's Hospital, Boston	Role of glycosaminoglycans in lung edema
IRWIN FRIDOVICH, Ph.D. *Professor of Biochemistry*, Duke University Medical Center, Durham, NC	Control of the biosynthesis of superoxide dismutases
ERROL C. FRIEDBERG, M.D. *Associate Professor of Pathology*, Stanford University, Stanford, CA	Complementing human cells with cloned yeast DNA repair genes
KJELL FUXE, M.D. *Professor of Histology*, The Karolinska Institute, Stockholm, Sweden	Nicotine, catecholamines, and neuroendocrine functions Smoking, dopamine, neuropeptides and models of Parkinson's disease
JACK GAULDIE, Ph.D. *Professor of Pathology*, McMaster University, Hamilton, Ontario, Canada	The mast cell in interstitial pulmonary fibrosis

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